EXHIBIT C

TITLE 32P-Kinasing of ChtA176

Project No.

Book No. 391

From Page No. —

Purpose - To label synthetic probe ChtA176 with ^{32}P by Kinasing for Chlamydia assay development.

Reagents -

Synthetic probe ChtA176 mm 320-39 1.28 OD/ml 64 µg/ml

10X Kinase buffer, from KB, mm, aliquot obtained (391:18)

T4 Kinase, BRL, 10 u/µl Lot 62111

γ-^{32}P-ATP 3000 Ci/mmol #G551, acd 10 µCi/µl

Teflon, acid-base treated, from Mark H.

5 m NaCl DEB NB 157:48

1% SDS diluted from 20% (DK)

1 M Tris, pH 8.2 (DK)

100 mM EE L/N 500 38

4 M LiCl from DK

Proteinase K 20 mg/ml 333:1

Glycogen HL-1 ~40 mg/ml in 10% EtOH (DK)

Phenol from DK ( )

Chloroform

10% TCA

BSA, 10 mg/ml from BRL Lot# 40416

Procedure

1. Follow same procedure used (391:18-20)
   Kinase reaction included 1 µl of ChtA176 (64 ng)

Results

Column rinses in 0.3 m NaCl and H_2O:

$\cong (9.6 \times 10^4)\left(\frac{3300}{5}\right) = 6.3 \times 10^7$ cpm

Straight count - $(6.2 \times 10^4)\left(\frac{200}{1}\right)\left(\frac{50}{10}\right) = 6.2 \times 10^7$ cpm > Why so low?

Input dpm = 2.2×10^8 dpm

EtOH super - $(1.1 \times 10^3)\left(\frac{2400}{5}\right) = 5.3 \times 10^5$ cpm

To Page No. 26

Witnessed & Understood by me,	Date	Invented by	Date
Joan Kop		Margaret Harper	
		Recorded by	

From Page No. 25

Results, cont.

TCA ppt able cpm = $(1.2 \times 10^4)\left(\frac{200}{1}\right)\left(\frac{50}{10}\right) = 1.2 \times 10^7$ cpm in 64 ng

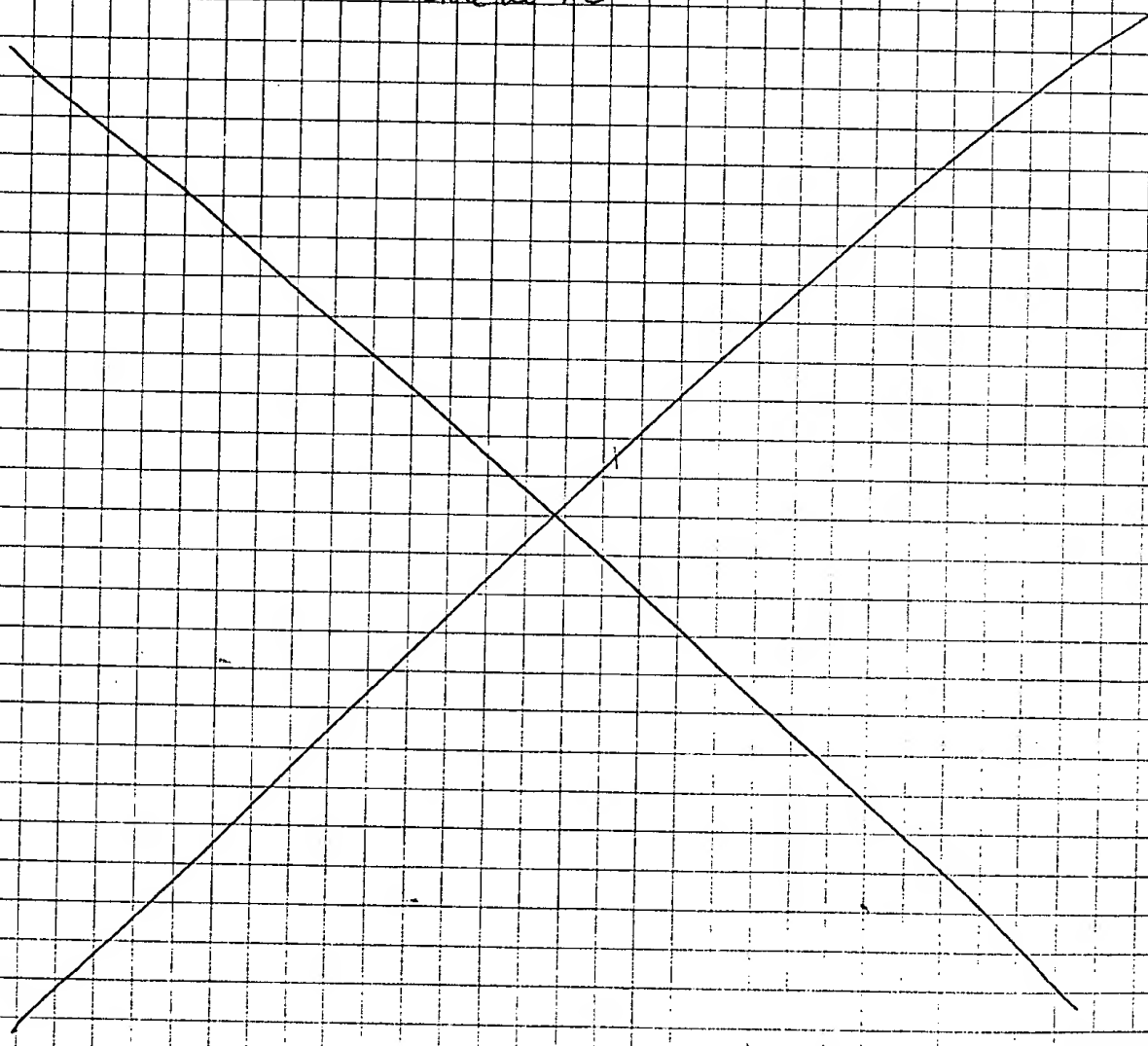
specific act. = 1.9×10^8 cpm/ug

Final cpm $(1.8 \times 10^5)\left(\frac{100}{2}\right) = 9 \times 10^6$ cpm in 100 ul H_2O — 75% recovery

9×10^4 cpm/ul

Diluted total sample to 300 ul 0.02% SDS → 3×10^4 cpm/ul final

store at 4°C



To Page No. —

Witnessed & Understood by me,	Date	Invented by	Date
joann Kop		Mary E. Hasper	
		Recorded by	